Pak. J. Bot., 45(6): 2093-2094, 2013.

PHYTOCHEMICAL STUDIES ON MANGROVE *AVICENNIA MARINA*

S.A. MAHERA[1*], S.M. SAIFULLAH[1], V.U. AHMAD[2] AND F.V. MOHAMMAD[2]

[1]*Department of Botany, University of Karachi, Karachi-75270, Pakistan*
[2]*H. E. J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi, Pakistan*
[*]*Corresponding author's e-mail: maherasyedali@yahoo.com*

Abstract

Stigmasterol-3-*O*-β-D glucopyranoside (**1**) has been isolated from pnematophores (aerial roots) of *Avicennia marina* (Forssk.) Vierh. along with two triterpenoids, ursolic acid (**2**) and α-amyrin (**3**). Compounds **1** and **2** are reported for the first time from this plant. Compound (**1**) was found to possess moderate anti-glycation activity.

Introduction

The genus *Avicennia* belongs to mangrove family *Avicenniaceae* which is widely distributed in all intertidal areas (Robertson & Alongi, 1992). The plants absorb oxygen through pnematophores, which is deficient in its habitat (Little, 1983). The knowledge about compounds derived from mangrove is very scarce but many important classes of compounds like alkaloids, benzofurans flavonoids, benzoquinones, tannins, triterpenes, aminoacids, carbohydrates, carotenoids, steroids, organicacids, glycoside, anthocyanides, procyanides, alcohols, sugars, lipids and nitrogen containing salts have previously reported (Bandaranayake, 2002).

The extracts of *Avicennia marina* from leaves and aerial parts have been reported to possess antiviral (Zandi *et al.*, 2009) and anti-microbial activities (Bobbarala *et al.,* 2009). Its bark and roots are used as aphrodisiac, the wood of plant is used for the treatment of snakebites and the extract of the seed for sores. Young fruits are used as poultice for wounds and leaves for skin ailments (List & Horhammer, 1979). The present study was undertaken to obtain more information about secondary metabolites of *A. marina.* We report herein the isolation and structure elucidation of a sterol glucoside along with two triterpenoids. The sterol glucoside showed moderate antiglycation activity.

Materials and Methods

Pneumatophores (aerial roots) of grey mangrove *A. marina* were collected from the backwater of Karachi Harbour known as Sandspit. The shaded-dried roots (6Kg) were extracted with methanol at room temperature. For column chromatography (CC), silica gel (70-230 mesh) and for flash chromatography (FC), silica gel (230-400 mesh) were used. TLC was performed on pre-coated silica gel G-25-UV 254 plates. Detection was carried out at 254nm, and spraying with Ceric sulphate reagent. H-NMR, ^{13}CNMR, Cosy-45, HMQC and HMBC spectra were run on Bruker Spectrometers operating at 500, 400 and 300MHz. The chemical shifts are given in ppm and coupling constants in Hz. HR- EI-MS and FAB spectra were recorded on a JMS-HX-110 spectrometer, with a data system.

The extract was subjected to CC over silica gel eluting with mixtures of hexane- ethyl acetate and ethyl acetate-methanol in increasing order of polarity. The fractions which eluted with hexane-ethyl acetate (2:1 and 1:1) showing similar TLC profile were combined and subjected to FC over silica gel. Elution with hexane-ethyl acetate (8:2) provided α-amyrin (3). Elution with hexane-ethyl acetate (6:4) furnished ursolic acid (2). Elution of the column with ethyl acetate –methanol (9.5: 0.5) provided a semi pure compound. It was subjected to FC and eluted with chloroform-methanol (9:1) to obtain stigmasterol-3-*O*-β-D glucopyranoside (1).

Antiglycation assay: It is a nucleophilic reaction in which protein amino group or lipid molecule is covalently linked with the carbonyl group of reducing sugar such as glucose and fructose to form glycated product called Glycation, Glycosylation, Non-enzymatic reaction or Advance glycated ends product (AGEs). It is posttranslational modification of protein which occur by hyperglycemia and long term complication such as Cataract,Neuropathy, Nephropathy, Wound healing, Alzheimer's disease (AD) etc. functioning of biomolecules. The method of anti-glycation activity is previously published. (Mahera *et al*., 2011)

Results and Discussion

The methanolic extract of the aerial roots of *A. marina* resulted in the isolation of compound 1 (Fig. 1) isolated for the first time from this plant. The FAB MS showed the $(M-H)^+$ at m/z 575.4239 which corresponded to the molecular formula ($C_{35}H_{58}O_6$, calculated 575.4239) by six degrees of un-saturation. The ^{1}H- NMR ($CDCl_3$,600MHz) of compound 1 presented a downfield broad singlet at δ5.36 which was assigned to the C-6 olefenic proton the C-3 proton resonated as a double doublet at δ 3.65 (*J*=11.9Hz, *J*=5.1 Hz). The 3H singlets at δ0.94, 0.93, 0.87, 0.85, 0.83, and 0.71 were assigned to the C-21, C-19, C-29, C-26, C-27 and C-18 methyl protons, respectively. The C-1 anomeric proton appeared as a doublet at δ 4.37 ($J_{1,2}$ =7.7Hz) showing the presence of a β sugar. The compound was identified as stigmasterol-3-*O*-β-D glucopyranoside (Alam *et al*., 1996) on comparison with the reported data.

The anti-glycation activity of compound **1** (Table 1) was carried out and showed moderate activity.

Table 1. Anti-glycation activity of compound 1.

Name of compound	% Inhibition
stigmasterol-3-O-β-D- glocopyranoside	58.5%
Rutin (standard)	85.5%

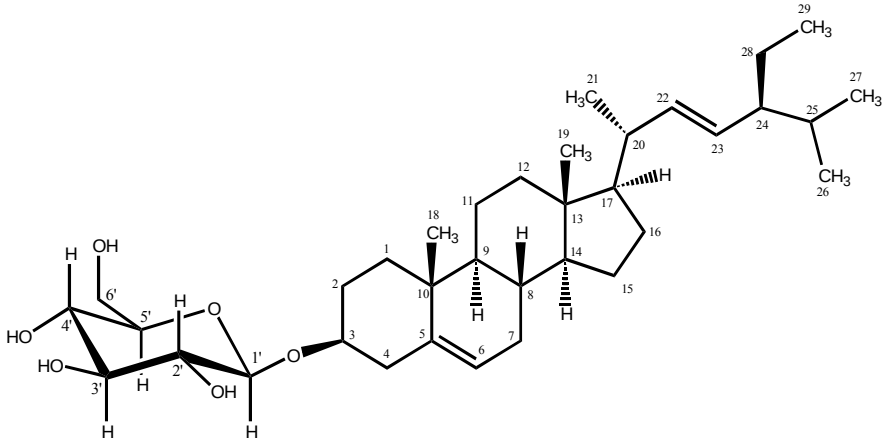

Fig. 1. Stigmasterol-3-O-β-D-glocopyranoside.

The compound 2 (Fig. 2) was isolated in crystalline form .The IR spectrum of **2** showed absorption for hydroxyl group (3510 cm^{-1}), carboxyl group (1697 cm^{-1}) and tri-subustituted double bond (1635 and 820 cm^{-1}). The molecular formula $C_{30}H_{48}O_3$ was recognized through HR-EIMS which showed molecular ion peak at m/z 456.3099(calculated for $C_{30}H_{48}O_3$. 456.3603). On the other hand, the HR-EIMS showed a peak at m/z 411. 3640 indicating the loss of -COOH group. Another major peak at m/z 248.1743 represented RDA fragmentation characteristic of Δ^{12} ursane type triterpenes with -COOH group at C-17 (Budzikiewicz *et al*., 1963). The compound was identified as ursolic acid (Siddiqui, *et al*., 1988) in comparison with the reported data.

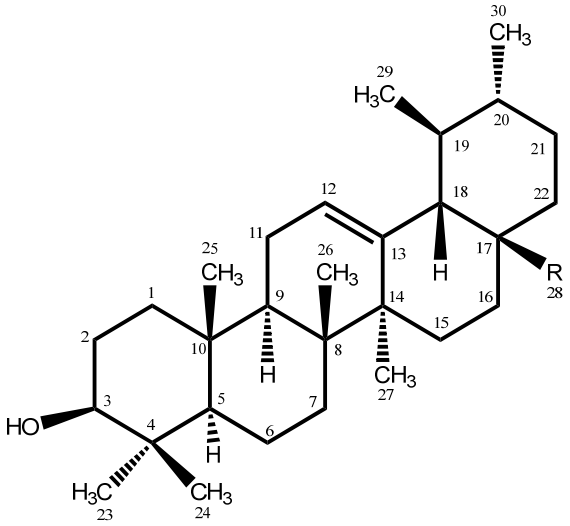

Fig. 2. Ursolic acid α-amyrin
R= CooH, R= CH_3

Compound 3 (Fig. 2) was isolated as amorphous solid from n-hexane fraction of methanol extract of *Avicennia marina*. The HREI MS of 3 showed the (M^+) ion at m/z 426.3852, according to the formula $C_{30}H_{50}O$.The spectral data were also found to be identical to the previously reported data. (Jares & Pomilio, 1987).

Acknowledgement

The authors are extremely thankful to the Director of H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi for providing facilities to carry out this research.

References

Alam, M.S., N. Chopra, M. Ali and M. Niwa. 1996. Oleanen and stigmasterol derivatives from *Ambroma augusta*. *Phytochemistry*, 41: 1197-1200.

Bandaranyake, W.M. 2002. Survey of mangrove plants from northern Australia for phytochmical constituent and UV-absorbing compound. Cure. *Topics in phytochem. Life Sci. Adv*., 14: 69-78.

Bobbarala, V., V. R. Vadlapudi and K.C. Naidu. 2009. Antimicrobial Potentialities of Mangrove Plant *Avicennia marina. JPR*, 2: 1019-1021.

Budzikiewicz, H., J.M. Wilson and C. Djerassi. 1963. Mass spectrometry in structural and stereochemical problems. XXXII.[1] Pentacyclic Triterpenes. *J. Am. Chem. Soc*., 85: 3688-3699.

Jares. E. and A.B. Pomilio. 1987. Pyrrolizidine alkaloids and other components. *J. Nat. Prod*., 50: 514.

List, P.H. and L. Horhammer. 1979. Hager handbuch der pharmazeutisehen prexis. Vols 2-6. *Springer-Verlag, Berlin.*

Little, E.L. 1983. Common fuelwood crops: a hand book for their identification. McClain Printing Co., Parsons, WV.

Mahera, S.A., S.M. Saifullah, V.U. Ahmad, F.V. Mohammad and K. Ambreen. 2011. Steroids and triterpenoids from grey mangrove *Avicennia marina. Pak. J. Bot*., 43(2): 1417-2011.

Robertson, A.I. and D.M Alongi. Eds. 1992. Tropical mangrove ecosystems. coastal and estuarine studies 41. *American Geophys-ical Union, Washington, DC*, p. 329.

Siddique, S., F. Hafeez, S. Begum and B.S. Siddique. 1988. Oleanderol, a new pentacyclic triterpene from the leave of *Nerium oleander. J. Nat. Prod*., 51: 229-233.

Zandi, K., M.T. Aherzadeh, R. Yaghoubi, S. Tajbakhsh, Z. Rastian, M. Fouladvand and K. Sartavi. 2009. Antiviral activity of *Avicennia marina* against herpes simplex virus type 1 and vaccine strain of poliovirus. *JMPR,* 3: 771-775.

(Received for publication 15 March 2011)